Patent Office 9/719245

GB 99/01848

INVESTOR IN PEOPLE

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

REC'D 19 JUL 1999
WIPO PCT

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed

Dated 8 July 1999

Patents Form 1/ / /

5 Act 1977
6)

The Patent Office

10 JUN 1998

10JUN98 E366692-5 D02894
P01/7700 25.00 - 9812376.3 The Patent Office

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

10 JUN 1998

Cardiff Road
Newport
Gwent NP9 1RH

1. Your reference

P21993/CPA/RMC

2. Patent application number
(The Patent Office will fill in this part)

9812376.3

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

The Queen's University of Belfast
8 Malone Road
BELFAST
BT9 5BN

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention

"Peptide"

5. Name of your agent *(if you have one)*

Murgitroyd & Company

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

373 Scotland Street
GLASGOW
G5 8QA

Patents ADP number *(if you know it)*

1198013

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d.)

Yes

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	6
Claim(s)	3
Abstract	
Drawing(s)	

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature Murgitroyd & Company Date 9 June 1998
Murgitroyd & Company

12. Name and daytime telephone number of person to contact in the United Kingdom: Roisin McNally, 0141 307 8400

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) *If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.*

b) *Write your answers in capital letters using black ink or you may type them.*

c) *If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.*

d) *If you have answered 'Yes' Patents Form 7/77 will need to be filed.*

e) *Once you have filled in the form you must remember to sign and date it.*

f) *For details of the fee and ways to pay please contact the Patent Office.*

"Peptide"

The present invention relates to a modified analogue of the signal peptide sequence from Karposi syndrome fibroblast growth factor (kFGF) to be used as a cell-permeant vehicle for the intracellular delivery of covalently linked anti-sense peptide nucleic acid sequences (PNAs).

PNAs have potential uses as antisense molecules for the control of gene expression. Since they are capable of binding tightly to DNA and RNA targets thus preventing DNA transcription to RNA and RNA translation to protein. These molecules thus have two potential uses of commercial importance:

1. As research reagents where scientists use antisense strategies to ablate selected genes in order to understand their function.

2. As pharmaceutical compounds for companies seeking to develop nucleic acid-based therapies.

Conventional anti-sense oligonucletide in vivo delivery is highly inefficient, even if long-lasting, less polar

phosphorothioates are used.

It is an object of the present invention to use cell permeable peptide import (CPPI) to deliver PNAs to live target cells.

Use of conventional oligonucleotides is being reduced due to the development of PNAs (Neilsen, et al., 1991), which are much more stable, being resistant to enzymic degradation (Jordan, et al., 1997). PNAs replace the phosphodiester backbone of nucleic acid with repeating N-(2-aminoethyl)glycine units to which natural nucleobases are attached through methylenecarbonyl linkers. Although more stable, PNAs suffer from similar accessibility problems as phosphorothioates do, and passive diffusion of unmodified PNA across lipid membranes is not efficient (Wittung, P., et al., 1995).

A small number of native peptide sequences can translocate across membranes of living cells in an energy-independent and receptor-independent manner. These peptides have been used to import active cargo into the cell. For example a peptide from the homeodomain of *Antennapedia* has been successfully used to import both peptidal inhibitors of protein kinase C (Theodore, et al., 1995) and conventional anti-sense oligonucleotides (Allinquant, et al., 1995).

The present invention provides use of cell permeable peptide import (CPPI) to deliver peptide nucleic acids (PNAs).

The present invention provides use of the signal peptide sequence from Karposi syndrome fibroblast growth factor (kFGF) for delivery of antisense peptide nucleic acid sequences (PNAs).

The invention provides modified peptide sequence I as detailed herein.

The invention also provides peptide sequences II and III as detailed herein.

The invention provides use of a peptide as defined herein together with lysine residues for multiple presentation of peptide nucleic acids.

The invention further provides use of peptides as defined herein together with lysine residues in the simultaneous presentation of different peptides nucleic acids.

The present invention combines the two above technologies to use CPPI to deliver PNAs to in vivo targets.

Example

In order to determine the best delivery system, a comparison of the ability of three different cell permeant peptides to accumulate in whole cells was undertaken. The three peptides (Table 1) were labelled with carboxyfluorescein and the amount accumulated intracellularly was assayed after exposure of cells to 50μM; peptide II = 0.4μM; peptide III = ~0.4μM.

Table 1

I	CFI	A A V A L L P A V L L A L L A P K K K
II	CFI	R F A R K G A L R Q K N V H E V K N
III	CFI	R P R P Q Q F O G L M r

Key Peptide I : modified kFGF signal sequence
Peptide II : PKC pseudosubstrate sequence
Peptide III : modified substance P
CFI : Carboxyfluorescein
Or : Ornithine
Boldface : Modifications to original sequence

Peptide I was modified to contain three lysines C-terminal of the hydrophobic signal sequence. This peptide, therefore, can accommodate three PNAs, each bonded to a lysine epsilon amino group. This can be extended using the Multiple Antigen Presentation (MAP) technology to present eight (or more) PNA's on one peptide I sequence. A 'lysine tree' constructed in this way accommodates eight copies of the same PNA (see Fig 1A), thus increasing the effective concentration delivered by each CPPI. Alternatively a carrier can be constructed containing three (or more) different PNAs directed towards different sites on the same target mRNA (see Fig. 1B). This strategy has been termed 'molecular triangulation' (Branch, A.D., 1998).

Fig. 1A - Multiple presentation of a single PNA species

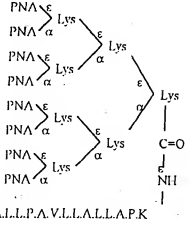
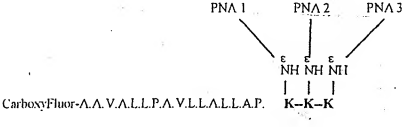


Fig. 1B - Simultaneous presentation of 3 PNAs directed to different sites on same target RNA



References

Allinquant, B., Hantraye, P., Mailleux, P., Moya, K., Bouillot, C. and Prochiantz, A (1995) Downregulation of amyloid precursor protein inhibits neurite outgrowth *in vitro* J. Cell Boil., 128: 919-927.

Branch, A.D. (1998) A good antisense molecule is hard to find. TIBS, 23: 45-50.

Jordan, S., Schwemler, C., Kosch, W., Kretschner, A., Schwenner, E., Stropp, U. and Mielke, B. (1997) Synthesis of new building blocks for peptide nucleic acids containing monomers with variations in the backbone. *Bioorg. Med. Chem. Lett.*, 7: 681-686.

Neilsen, P.E., Egholm, M., Berg, R.H. and Buchardt, O. (1991) Sequence-selective recognition of RNA by strand displacement with a thymine-substituted polyamide. *Science*, 254: 1497-1500.

Theodore, L. Derossi, D., Chassang, G., Llirbat, B., Kubes, M., Jordan, P., Chneiweiss, H., Godement, P. and Prochiantz, A. (1995) Intraneuronal delivery of protein kinase C pseudosubstrate leads to growth cone collapse. J. Neurosci., 15: 7158-7167.

Wittung, P., Kajanus, J., Edwards, K., Haaima, G., Nielson, P., Norden, B. and Malmstrom, B.G. Phospholipid membrane-permeability of peptide nucleic-acid (1995) *FEBS Lett.*, 375: 317-320.